

SEQUENCE LISTING

Reb D
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Yabuta, Masayuki
Suzuki, Yuji

<120> Process for Producing Peptides Using a Helper Peptide

<130> 001560-373

<140> US 09/402,093
<141> 1999-09-29

<150> PCT/JP99/00406
<151> 1999-01-29

<150> JP 10-32272
<151> 1998-01-30

<160> 24

<170> PatentIn version 3.0

<210> 1
<211> 4
<212> PRT
<213> Artificial Sequence

<220>

<223> Amino acid sequence adjacent to a site cleaved by enterokinase.

<400> 1

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Asp Asp Asp Lys
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<210> 2
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<212> PRT
<213> Artificial Sequence

<220>

<223> Amino acid sequence adjacent to a site cleaved by blood coagulation Factor Xa.

<400> 2

Ile Glu Gly Arg
1

<210> 3
<211> 7
<212> PRT
<213> Artificial Sequence

<220>

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<223> Amino acid sequence containing a site cleaved by renin.

<400> 3

Pro Phe His Leu **Leu** Val Tyr
1 5

<210> 4

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide.

<400> 4

Val Asp Asp Asp Asp Lys
1 5

<210> 5

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide.

<400> 5

Gly Cys His His His
1 5

<210> 6

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a chemically cleaved site.

<400> 6

Pro Gly Gly Arg Pro Ser Arg His Lys Arg
1 5 10

<210> 7

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide.

<400> 7

His Arg His Lys Arg Ser His His His

1 5 10

<210> 8
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence containing a site cleaved by Kex2 protease.

<400> 8

Ser Asp His Lys Arg
1 5

<210> 9
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence containing a position cleaved by OmpT.

<400> 9

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His
1 5 10 15

Arg Trp Gly Arg Ser Gly Ser
20

<210> 10
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<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence containing a position cleaved by OmpT.

<400> 10

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His
1 5 10 15

Gly Ser Gly Ser
20

<210> 11
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence coding for an amino acid sequence containing
a site cleaved by OmpT

<220>

Mark D

<221> CDS
<222> (1)..(69)

<400> 11
cag atg cat ggt tat gac gcg gag ctc cgg ctg tat cgc cgt cat cac 48
Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His
1 5 10 15

cgg tgg ggt cgt tcc gga tcc 69
Arg Trp Gly Arg Ser Gly Ser
20

<210> 12
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence containing a site cleaved by OmpT.

<400> 12

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His
1 5 10 15

Arg Trp Gly Arg Ser Gly Ser
20

<210> 13
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence coding for an amino acid sequence containing
a site cleaved by OmpT

<400> 13

tggttatgac gcggagctcc gcctgtatcg ccgtcatcac ggttccg

47

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<210> 14
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence coding for an amino acid sequence containing
a site cleaved by OmpT

<400> 14

gatccggaac cgtgatgacg gcgatacagg cggagctccg cgtcataacc atgca

55

Artificial

<210> 15
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer.

<400> 15
 gactcagatc ttcctgaggc cgat

24

<210> 16
 <211> 36
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer.

<400> 16
 aaaggtacct tccgcattgcc gcggatgtcg agaagg

36

<210> 17
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer.

<400> 17
 aggccagaa ccgtaaaaag

20

<210> 18
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer.

<400> 18
 aaaaatgcatac gcatcgtaac cgtgcatact

29

<210> 19
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Nucleotide sequence coding for a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

<220>
 <221> CDS
 <222> (82)..(543)

<400> 19
 cccaggctt acactttatg cttccggctc gatatgttg tggaatttgtg agcggataac 60
 aatttcacac aggaaacacgc t atg acc atg att acg gat tca ctg gcc gtc
 Met Thr Met Ile Thr Asp Ser Leu Ala Val
 1 5 10
 gtt tta caa cgt aaa gac tgg gat aac cct ggc gtt acc caa ctt aat 159
 Val Leu Gln Arg Lys Asp Trp Asp Asn Pro Gly Val Thr Gln Leu Asn
 15 20 25
 cgc ctt gca gca cat ccc cct ttc gcc agc tgg cgt aat agc gac gac 207
 Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp
 30 35 40
 gcc cgc acc gat cgc cct tcc caa cag ttg cgc agc ctg aat ggc gaa 255
 Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu
 45 50 55
 tgg cgc ttt gcc tgg ttt ccg gca cca gaa gcg gtg ccg gca agc ttg 303
 Trp Arg Phe Ala Trp Phe Pro Ala Pro Glu Ala Val Pro Ala Ser Leu
 60 65 70
 ctg gag tca gat ctt cct gac gcc gat act gtc gtc gtc ccc tca aac 351
 Leu Glu Ser Asp Leu Pro Asp Ala Asp Thr Val Val Val Pro Ser Asn
 75 80 85 90
 tgg cag atg cac ggt tac gat gcg atg cat ggt tat gac gcg gag ctc 399
 Trp Gln Met His Gly Tyr Asp Ala Met His Gly Tyr Asp Ala Glu Leu
 95 100 105
 cgc ctg tat cgc cgt cat cac ggt tcc gga tcc cct tct cga cat ccg 447
 Arg Leu Tyr Arg Arg His His Gly Ser Gly Ser Pro Ser Arg His Pro
 110 115 120
 cgf cat gcf gaa ggt acc ttt acc agc gat gtg agc tcg tat ctg gaa 495
 Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu
 125 130 135
 ggt cag gcf gca aaa gaa ttc atc gcf tgg ctg gtg aaa ggc cgt ggt 543
 Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
 140 145 150
 taagtcgaca gcccgcctaa tgagcggctc ttttttctc ggaatttaatt ctcatgtttg 603
 acaqcttatac atcqataaqc ttta 627

<210> 20
<211> 154
<212> PRT
<213> Artificial Sequence

<220>

<223> Amino acid sequence of a fusion protein comprising GLP-1,
helper peptide and beta-galactosidase protective peptide.

<400> 20

Met	Thr	Met	Ile	Thr	Asp	Ser	Leu	Ala	Val	Val	Leu	Gln	Arg	Lys	Asp
1				5					10				15		

Trp	Asp	Asn	Pro	Gly	Val	Thr	Gln	Leu	Asn	Arg	Leu	Ala	Ala	His	Pro
					20			25					30		

Pro	Phe	Ala	Ser	Trp	Arg	Asn	Ser	Asp	Asp	Ala	Arg	Thr	Asp	Arg	Pro
					35		40				45				

Ser	Gln	Gln	Leu	Arg	Ser	Leu	Asn	Gly	Glu	Trp	Arg	Phe	Ala	Trp	Phe
					50		55				60				

Pro	Ala	Pro	Glu	Ala	Val	Pro	Ala	Ser	Leu	Leu	Glu	Ser	Asp	Leu	Pro
					65		70			75			80		

Asp	Ala	Asp	Thr	Val	Val	Val	Pro	Ser	Asn	Trp	Gln	Met	His	Gly	Tyr
					85			90				95			

Asp	Ala	Met	His	Gly	Tyr	Asp	Ala	Glu	Leu	Arg	Leu	Tyr	Arg	Arg	His
					100			105				110			

His	Gly	Ser	Gly	Ser	Pro	Ser	Arg	His	Pro	Arg	His	Ala	Glu	Gly	Thr
							115		120				125		

Phe	Thr	Ser	Asp	Val	Ser	Ser	Tyr	Leu	Glu	Gly	Gln	Ala	Ala	Lys	Glu
					130		135			140					

Phe	Ile	Ala	Trp	Leu	Val	Lys	Gly	Arg	Gly
					145		150		

<210> 21
<211> 187
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence of a fusion protein comprising GLP-1, helper
peptide and beta-galactosidase protective peptide.

<400> 21

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp
 1 5 10 15

Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro
 20 25 30

Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro
 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe
 50 55 60

Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro
 65 70 75 80

Glu Ala Asp Thr Val Val Pro Ser Asn Trp Gln Met His Gly Tyr
 85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro
 100 105 110

Pro Phe Val Pro Thr Glu Pro His His His His Gly Gly Arg Gln
 115 120 125

Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Arg
 130 135 140

Trp Gly Arg Ser Gly Ser Pro Ser Arg His Lys Arg His Ala Glu Gly
 145 150 155 160

Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys
 165 170 175

Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
 180 185

B1
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<210> 22
 <211> 184
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Amino acid sequence of a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

<400> 22

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp
 1 5 10 15

Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro
 20 25 30

Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro
 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe

50 55 60
 Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro
 65 70 75 80
 Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr
 85 90 95
 Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro
 100 105 110
 Pro Phe Val Pro Thr Glu Pro His His His His Gly Gly Arg Gln
 115 120 125
 Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Gly
 130 135 140
 Ser Gly Ser Pro Ser Arg His Lys Arg His Ala Glu Gly Thr Phe Thr
 145 150 155 160
 Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile
 165 170 175
 Ala Trp Leu Val Lys Gly Arg Gly
 180

<210> 23
 <211> 184
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Amino acid sequence of a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

<400> 23

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp
 1 5 10 15

Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro
 20 25 30

Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro
 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe
 50 55 60

Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro
 65 70 75 80

Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr
 85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro
 100 105 110

Pro Phe Val Pro Thr Glu Pro His His His His His Gly Gly Arg Gln
115 120 125

Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Gly
130 135 140

Ser Gly Ser Pro Ser Arg His Pro Arg His Ala Glu Gly Thr Phe Thr
145 150 155 160

Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile
165 170 175

Ala Trp Leu Val Lys Gly Arg Gly
180

<210> 24

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a site cleaved by Kex2 Protease.

<400> 24

Ser Cys His Lys Arg
1 5